

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS P. CERRETTI

Appln. No. 08/538,709

Filed: October 3, 1995

For: DNA ENCODING CYTOKINE DESIGNATED
LERK-6



Group Art Unit: 1647

Examiner: Draper, G.

DECLARATION UNDER 37 C.F.R. § 1.131

Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

I, DOUGLAS P. CERRETTI do hereby declare and state:

I am the inventor of the invention disclosed and claimed in the above-mentioned application.

I am familiar with U.S. Patent 5,795,734, issued to Flanagan on August 18, 1998 (hereinafter the "Flanagan Patent"), from U.S. Patent Application Serial No. 08/455,001, filed May 31, 1995 (hereinafter the "Flanagan Application"), which I understand is a Continuation-In-Part of Serial No. 08/393,461, filed February 27, 1995 (hereinafter the "Flanagan Parent Application"), which is a Continuation-In-Part of Serial No. 08/308,814, filed September 19, 1994 (hereinafter the "Flanagan Grandparent Application").

The Flanagan Patent discloses DNA encoding Elf-1, a mouse polypeptide which is now known in the art as mouse LERK-6.

In order to demonstrate and establish, *inter alia*, that I conceived and reduced to practice a DNA molecule encoding a polypeptide having mouse LERK-6 sequences in the United States by at least September 15, 1994, i.e., prior to the

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September 19, 1994, filing date of the Flanagan Grandparent Application, copies of the following laboratory notebook pages and related materials, identified in detail below, are provided herewith in **Appendices A-G**.

I declare and state that although all of the dates from the laboratory notebook pages and related materials have been removed, all of the dates are prior to at least September 15, 1994.

Appendix A contains pages from Nicole Nelson's Laboratory Notebook No. 4266 (Bates Nos. 0001-0007). At the time, Nicole Nelson was a Research Assistant who worked under my direction and supervision at Immunex Corporation.

Appendix B contains a copy of U.S. Patent 5,516,658 (Bates Nos. 0008-0026).

Appendix C contains oligonucleotide request forms prepared by Carl Kozlosky (Bates Nos. 0027-0030). At the time, Carl Kozlosky was a Research Associate who worked under my direction and supervision at Immunex Corporation.

Appendix D contains pages from Carl Kozlosky's Laboratory Notebook No. 3388 (Bates Nos. 0031-0037).

Appendix E contains various computer printouts of LERK sequences that I personally generated (Bates Nos. 0038-0049).

Appendix F contains the minutes of an internal HEK/ELK meeting at Immunex Corporation that was chaired by Barry Davison in my absence (Bates Nos. 0050-0051). Barry Davison, who prepared the minutes, was the Director of the Transgenics Department at Immunex Corporation at the time.

Appendix G contains a copy of American Type Culture Collection Form BP4/9 for ATCC deposit No. 75829 (Bates No. 0052).

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Prior to September 15, 1994, and as described in Example 1 of the present application, a DNA molecule encoding mouse LERK-6 was isolated under my direction and supervision. The specific experiments detailed below were carried out at my behest and command and under my direction and supervision by scientists at Immunex Corporation.

More specifically, prior to September 15, 1994, and as described at page 23, lines 5-7 of the present application, a commercially available 11.5 day murine embryonic cDNA library was obtained by Nicole Nelson from Clontech Laboratories, Inc., Palo Alto, California (**Appendix A**, Bates No. 0003).

Next, prior to September 15, 1994, and as described on page 23, lines 7-8 of the present application, the library was plated by Nicole Nelson according to the procedures detailed in the manual provided by Clontech (**Appendix A**, Bates No. 0004). The initial purpose of these efforts was to clone the cDNA for mouse LERK-3 (referred to as A2) and mouse LERK-4 (referred to as C6). However, instead a new mouse LERK molecule was discovered, i.e., mouse LERK-6.

Prior to September 15, 1994, and as described on page 23, lines 8-30 of the present application, probes, referred to as A2 (LERK-3) and C6 (LERK-4), were generated using standard techniques. Generally, polymerase chain reaction (PCR) (Mullis et al, *Meth. Enzymol.*, 155:335-350 (1987)) amplifications were performed by Carl Kozlosky (**Appendix D**, Bates Nos. 0036-0037) using two sets of primers. The first set of primers,

GATATTTACT GCCCGCACTA CAACAGCT

SEQ ID NO:3

AGAGAAGGCG CTGTAGCGCT GGAAC

SEQ ID NO:4

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was used to generate amplified double stranded DNA fragments from the DNA of LERK-3 (LERK-3, also known as hek-ligand, is the subject of U.S. Patent 5,516,658 (**Appendix B**, Bates Nos. 0008-0026) which issued from U.S. Patent Application Serial No. 08/240,124, filed May 9, 1994, and which claims benefit of Serial Nos. 08/109,745, 08/114,426 and 08/161,132). The probe from LERK-3 comprised nucleotides 260 through 481 of the SEQ ID NO:1 of U.S. Patent 5,516,658. The second set of primers,

ACGTAGTCTA CTGGAAGTCC AGTAACCCCA G SEQ ID NO:5

AGCCTCAAGC ACTGGCCAGA ACTCTCTCTG GAGT SEQ ID NO:6

was used to generate amplified double stranded DNA fragments from the DNA of LERK-4 (LERK-4 also is the subject of U.S. Patent 5,516,658). The probe from LERK-3 comprised nucleotides 110 through 467 of the SEQ ID NO:3 of U.S. Patent 5,516,658.

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:3, i.e., oligo #12334 (also referred to as A2rib5.28) (**Appendix C**, Bates No. 0027), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0027).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:4, i.e., oligo #12333 (also referred to as A2T7.49) (**Appendix C**, Bates No. 0023), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0023).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:5, i.e., oligo #12312

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(also referred to as C6RIBO5.31) (**Appendix C**, Bates No. 0029), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0029).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:6, i.e., oligo #12316 (also referred to as C6T7.54) (**Appendix C**, Bates No. 0030), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0030).

Oligonucleotides #12333 and 12316 also included nucleotides encoding the T7 polymerase promoter.

Prior to September 15, 1994, Carl Kozlosky identified the location of oligonucleotides #12334 (A2rib5.28) and #12333 (A2T7.49) in the LERK-3 DNA sequence, as shown in **Appendix D**, Bates Nos. 0032-0033); as well as the location of oligonucleotides #12312 (C6RIBO5.31) and 12316 (C6T7.54) in the LERK-4 DNA sequence, as shown **Appendix D**, Bates Nos. 0034-0035).

Thereafter, and prior to September 15, 1994, and as described on page 23, lines 30-35 of the present application, the PCR fragments (A2/LERK-3 and C6/LERK-4) were radiolabelled by Nicole Nelson with ³²P (**Appendix A**, Bates No. 0005), and used by Fred Fletcher as probes to screen the murine embryonic cDNA library prepared by Nicole Nelson by conventional procedures, and hybridizing clones were identified. Fred Fletcher was a Staff Scientist at Immunex Corporation at the time who worked on the LERK project under my direction and supervision. The hybridizing conditions consisted of 42°C and 50% Starks washed to 0.1X SSC at 63°C (**Appendix A**, Bates Nos. 0005-0006). In this manner, clone #13 was identified by Fred Fletcher on an X-ray

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film, a copy of which was placed in Nicole Nelson's Laboratory Notebook (**Appendix A**, Bates No. 0007).

Then, and prior to September 15, 1994, and as described on page 23, line 35 and page 24, line 4, of the present application, the nucleotide sequence of the cDNA insert of clone #13 (λ 13), isolated from the murine embryonic cDNA library, i.e., mouse LERK-6 (mLERK6), was determined at my request by the core facility at Immunex Corporation, and the results were entered into a computer database, a printout of which, which was generated prior to September 15, 1994, is shown in **Appendix E**, Bates Nos. 0038-0039. DNA encoding the first 5 amino acids shown in **Appendix E** is derived from the sequencing vector, as indicated by the mark between the fifth amino acid (Arg) and the sixth amino acid (Ala). Also, the initiation codon Met is not shown in **Appendix E**. Thus, a substantially complete cDNA sequence of the coding region of the clone λ 13 cDNA, and the amino acid sequence encoded thereby were determined, and are presented in SEQ ID NO:1 and SEQ ID NO:2, respectively of the present application. The open-reading frame within this sequence in **Appendix E** (and within SEQ ID NO:1) encodes a protein of 184 amino acids beginning with the second Ala.

Prior to September 15, 1994, I carried out a comparison of the amino acid sequences of mouse LERK-6 v. human LERK-3 (also referred to as A2) (**Appendix E**, Bates No. 0040); mouse LERK 6 v. human LERK-4 (also referred to as C6) (**Appendix E**, Bates No. 0041); mouse LERK 6 v. human LERK-2 (also referred to as ELKL) (**Appendix E**, Bates No. 0042); mouse LERK-6 v. human LERK-5 (**Appendix E**, Bates No. 0043); mouse LERK-6 v. human LERK-1 (also referred to as B61) (**Appendix E**, Bates No. 0044); mouse LERK-6 v. mouse LERK-4 (also referred to as MC6) (**Appendix E**, Bates

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No. 0045); as well as the DNA sequences for mouse LERK-6 v. human LERK-3 (A2) (**Appendix E**, Bates Nos. 0046-0047),); mouse LERK-6 v. mouse LERK-4 (MC6) (**Appendix E**, Bates No. 0048); and mouse LERK-6 v. human LERK-5 (**Appendix E**, Bates No. 0049). These comparisons showed many conserved amino acids and nucleotides amongst the family, and clearly showed that LERK-6 was a member of the LERK family of proteins, and thus would bind to hek/elk.

Prior to September 15, 1994, the results of the above-discussed experiments were presented by Nicole Nelson at an internal HEK/ELK meeting at Immunex Corporation. This meeting was chaired by Barry Davison in my absence, who prepared the meeting minutes, a copy of which is shown in **Appendix F** Bates No. 0050 0051.

Next, as described on page 24, lines 14-17 of the present specification, on July 15, 1994, a cell lysate containing clone λ 13 DNA (the LERK-6 cDNA in λ gt10) was deposited with the American Type Culture Collection, Rockville, MD, USA, and assigned accession number ATCC 75829. A copy of the deposit receipt is shown in **Appendix G**, Bates No. 0052.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date:

2/20/01

Name:

Douglas P. Cerretti
DOUGLAS P. CERRETTI

NOTEBOOK NO. 7866
ISSUED TO Nicole Nelson
ON _____ 19____
DEPARTMENT Ad. Div.
RETURNED _____ 19____

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

0001

IMMUNEX LABORATORY NOTEBOOK

"TABLE OF CONTENTS" FORM

Notebook #: 1266 Date form completed:

Form Completed by: David Nelson

MOLECULE(S): C MGF

~~HEP~~

HEP

AIK

LEAK 4

LEAK 3

PROJECT(S): _____

Product Analysis Certificate

PRODUCT: Mouse Embryo 5'-STRETCH cDNA Library

CAT. #: ML1027a

LOT #: 1211

STORAGE CONDITIONS:

SHORT-TERM STORAGE (< 6 MONTHS)
4°C

LONG-TERM STORAGE (> 6 MONTHS)
-70°C

SHELF LIFE:

1 year from date of receipt under
proper storage conditions

SHIPPING CONDITIONS:

Dry Ice (-70°C)

PACKAGE CONTENTS:

- 0.2 ml library lysate in 1X Lambda Dilution Buffer and 7% DMSO
- 0.5 ml host strain
- Lambda Library Protocol Handbook (PT1010)

TITER: $\geq 10^6$ pfu/ml

CLONING VECTOR: λ gt10

CLONING SITE: *EcoRI*

PRIMING METHOD: oligo(dT)-primed

HOST STRAIN: C600 *Hfl*

mRNA SOURCE:

whole embryo (not including placenta
extraembryonic membranes) from a cross between
ICR outbred females and outbred Swiss Webster
males, 11.5 days post-coitus (noon on the day
vaginal plug is 0.5 day post-coitus)

NOTE: No further information on the mRNA source
was made available to CLONTECH.

QUALITY CONTROL DATA

SELECTION CRITERIA: Clear plaques from turbid plaques (nonrecombinant
or parental)

ESTIMATED
% OF CLEAR PLAQUES: 86%

NUMBER OF
INDEPENDENT CLONES: 1.7×10^6
(when plated on C600 before amplifying in C600 *Hfl*)

AVERAGE INSERT SIZE: 1.5 kb

INSERT SIZE RANGE: 0.8-4.0 kb

AMPLIFICATION: This library was amplified once in C600 *Hfl*.

APPROVED BY:

(PA93650-1)

0003

FOR RESEARCH USE ONLY

Page No.

Titer library

2d library

1.5 x 10⁵

1.25 x 10⁵

0.1

2.1

3.2

4.2

5.2

6.2

7.2

8.2

9.2

10.2

11.2

12.2

13.2

14.2

15.2

16.2

17.2

18.2

19.2

20.2

21.2

22.2

23.2

24.2

25.2

26.2

27.2

28.2

29.2

30.2

31.2

32.2

33.2

34.2

35.2

36.2

37.2

38.2

39.2

40.2

41.2

42.2

43.2

0.1

2.1

3.2

4.2

5.2

6.2

7.2

8.2

9.2

10.2

11.2

12.2

13.2

14.2

15.2

16.2

17.2

18.2

19.2

20.2

21.2

22.2

23.2

24.2

25.2

26.2

27.2

28.2

29.2

30.2

31.2

32.2

33.2

34.2

35.2

36.2

37.2

38.2

39.2

40.2

41.2

42.2

43.2

Plate the cells at 5 x 10⁴

5 x 10⁴ cells at 1.5 x 10⁵

5 x 10⁴ cells at 1.25 x 10⁵

2d library

1.5 x 10⁵

1.25 x 10⁵

1.5 x 10⁵

1.25 x 10⁵

5 x 10⁴ cells at 3.75 x 10⁴ per plate

Take a fresh dilution and plate at 3.75 x 10⁴ per plate (2) - plated by 10.4.4

The plates did not grow in 8 hours I left them overnight. They had grown to full size & the concentration was 10 fold less.

0004

To Page No. 87

essed & Understood by me,

Date

Invented by

Date

DNL

Recorded by *David Nelson*

TITLE 2. Search for a new geneFrom Page No. 85

PROGRAM # = 13 2X 'Prime It' then cleared a 2 650 columns 01:44
 REGION A: LL-UL = 5-1700 LCR = 0 BKG = .00 % 2 SIGMA = .0
 REGION B: LL-UL = 50-1700 LCR = 0 BKG = .00 % 2 SIGMA = .0
 REGION C: LL-UL = 0-0 LCR = 0 BKG = .00 % 2 SIGMA = .0
 TIME = 1.00 K = 1.00 QIP = SIS

P#	S#	TIME	CPMA/K	%DEV	CPMB/K	%DEV	CPMC/K	%DEV	SIE	SIS	FLAGS	MIN
WARNING: NOT NORMALIZED												
13	1	1.00	481546.	.29	9757.00	2.02	.00	.00	.000	41.366	180	112
13	2	1.00	666133.	.25	22258.0	1.34	.00	.00	.000	45.390	110	306

PROGRAM # = 13 23:32
 REGION A: LL-UL = 5-1700 LCR = 0 BKG = .00 % 2 SIGMA = .0
 REGION B: LL-UL = 50-1700 LCR = 0 BKG = .00 % 2 SIGMA = .0
 REGION C: LL-UL = 0-0 LCR = 0 BKG = .00 % 2 SIGMA = .0
 TIME = 1.00 K = 1.00 QIP = SIS

P#	S#	TIME	CPMA/K	%DEV	CPMB/K	%DEV	CPMC/K	%DEV	SIE	SIS	FLAGS	MIN
WARNING: NOT NORMALIZED												
13	1	1.00	389178.	.32	6839.00	2.42	.00	.00	.000	37.510	120	112
13	2	1.00	293853.	.37	4696.00	2.92	.00	.00	.000	35.396	120	306

A2 4.7×10^7 total cutsC6 3.5×10^7 total cuts

Maxine cDNA library for HEK cells A2

These probes worked well on the positive controls included in the hybridization. See Fred Fletcher for films.

0005

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Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

1.94

NIH embryo
cDNA library

probed w/ A2, C6
and GSK- β .

probed 42°C on stalks

washed to .1xSSC, 63°

1° films

from Fred Fletcher

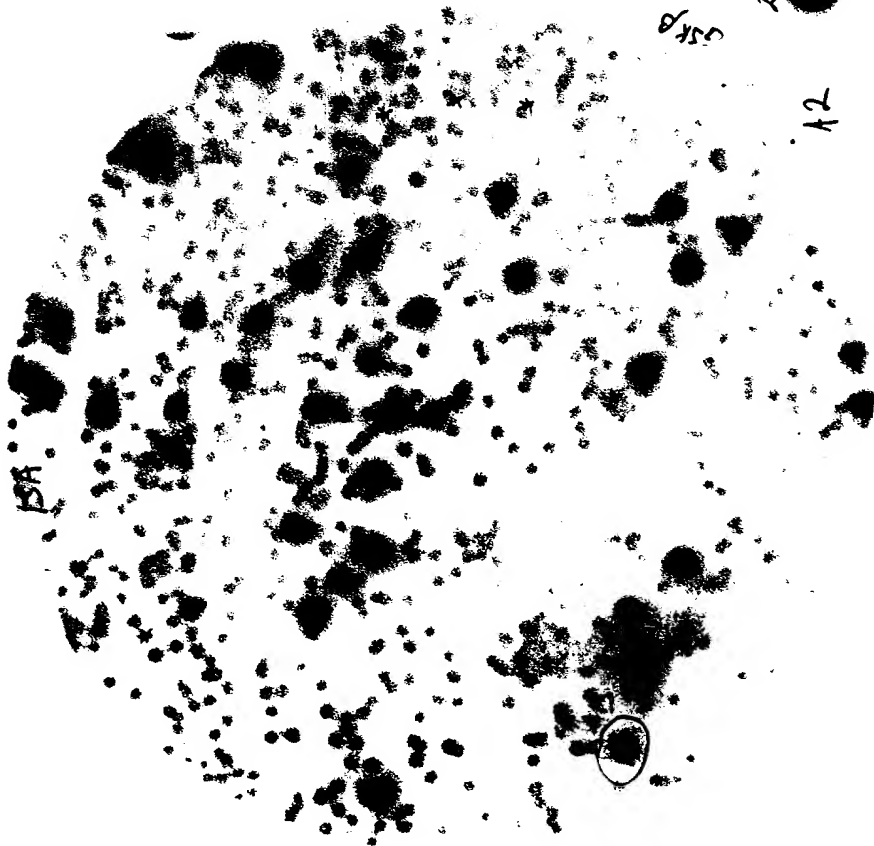
I plated this library before Xmas 93
but received these films

0006

Mu. DNA 2°

P. hyb 42° 12 hrs
 Hyb 42° 9h 50% STARKS 120000 120000 120000
 Wash AT 6X SSC 12.5DS 10' 60'
 63° 1X SSC 10' 20'
 65° 0.1X SSC 10' 20'

exp 3097 4264/87



200 100 1019

250 100 5 17

250 100 5 07

12

16

Oligo Name:

A2RIB5.28

Sequence Requested by:

KOZLOSKY

Project name:

ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'):

5'-GAT ATT TAC TGC
CCG CAC TAC AAC AGC
T-3'

PURIFICATION: PHENOL

(2R base)

8A's 4G's
9C's 7T's

COMMENTS:

A2 5 PRIME PCR OLIGO FOR
MAKING A 77 RIBOPROBE.

A2rib5.28

R7043

1 GATATTTACT GCCCGCACTA CAACAGCT

Column 2

9:44:32A

Run ID :

Cycle : 40PLUS CYC

End Frpc: End CE

(DMT = On)

Sequence: 12334

Total bases = 28

A= 8, G= 4, C= 9, T= 7, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 8489.6

5' GAT ATT TAC TGC CCG CAC TAC AAC AGC T <3'

Purification:

Amount of crude:

O.D.260:

dilution factor:

concentration:

yield:

OPC

all

1.600

1:500

10.09 µg/λ

100.9 µg

gel on
back

0027

Oligo NAME: A2T7.49

Oligo number: 12333

Sequence Requested by: KOZLOSXY
Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-TGC GAA TAA TAC
GAC TCA CTA TAG AGA
GAA GGC GCT GTA
CTG GAA C-3'

PURIFICATION: PHENOL

(49 bases)

16A's 14G's
10C's 9T's

COMMENTS:

3 PRIME A2 OLIGO TO PCR
A T7 RIBOPROBE. THIS
OLIGO IS ANTISENSE AND
CONTAINS THE T7
PROMOTER.

A2t7.49

R7044

1 TGC GAATAAT ACGACTCACT ATAGAGAGAA GGCGCTGTAG CGCTGGAAAC

Column 1

9:44:31A

Run ID :

Cycle : 40PLUS CYC

End Proc: End CE

(DMT = On)

Sequence: 12333

Total bases = 49

A= 16, G= 14, C= 10, T= 9, S= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 15174.8

5' > TGC GAA TAA TAC GAC TCA CTA TAG AGA GAA GGC GCT GTA

GGC CTG GAA C <3'

Purification:

Amount of crude:

O.D.260:

dilution factor:

concentration:

yield:

OPC

all

276

1:500

4.60 µg/λ

460 µg

gel on
12334

0028

Oligo NAME: C6RIB05.31

Oligo number:

Sequence Requested by: KOZLOSKY
Project name: ELK

12312

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'):
5'-ACG TAG TCT ACT
GGA ACT CCA GTA ACC
CCA G-3'

(31 bases)

9A's 6G's
10C's 6T's

PURIFICATION: ~~standard~~ OPL

COMMENTS:
5 PRIME PCR FOR C6 RIBO

R7023

121400.2

0:43:43F

OT ID:

File: 40FLU2.D

End Proc: End CE

DMT = On

Bo
Sequence: 12312

eb Applied Biosystems G 209118

otal bases = 31

A= 5, G= 6, C= 10, T= 6, 5= 0, 6= 0, 7= 0, 8= 0
mixed bases= 0

W: 9444.2

5'-ACG TAG TCT ACT GGA ACT CCA GTA ACC CCA G-3'

Purification: OPL

Amount of crude: all

O.D.260: 0.382

dilution factor: 1-500

concentration: 6.36 ug/lx

yield: 636 ug

get on
12,334

0029

Oligo NAME: C6T7.54
Sequence Requested by: KOZLOSKY
Project name: ELF

Oligo number: 12316

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-TGC GAA TAA TAC
GAC TCA CTA TAG CCT
CAA GCA CTG GCC AGA
ACT CTC TGG AGT -3'

(54 bases)

16A's 11G's
15C's 12T's

PURIFICATION: ~~PHENOL~~ OPC

COMMENTS:
C6 3 PRIME FOR C6 RIBO
USE T7 POL.



R7024

COLUMN 2 SET-UP
VERSION 2.02

USER_NAME:
CYCLES USED: 0.20MB - 1
ENDING METHOD: Trityl ON, Auto
ENDING PROCEDURE: deprime
SEQUENCE NAME: 12316
SEQUENCE LENGTH: 54
DATE:
TIME: 17:37
COMMENT:

5'- TGC GAA TAA TAC GAC TCA CTA TAG CCT CAA GCA CTG

GCC AGA ACT CTC TGG AGT -3'

yield:

OPC
all
0.303
1.500
5.04 ug/1x
504 ug

gel on
12334

0030

IMMUNEX LABORATORY NOTEBOOK

"TABLE OF CONTENTS" FORM

Notebook #: 3388

Date form completed:

Form Completed by: Carl Kozlosky

MOLECULE(S): B61, ELK, ELK-L, HK,
LeKs 1, 2, 3, 4, 5, 7

PROJECT(S): RISOL

With 114 enzymes: *

T7 is 5' End
Bg12 into Bam HI

09:13 ..

X E B B B N
 h s s s s s
 o P 3 P B1 2
 2 1 P M 1 26 1 2

1 GGATCTTGGAAACGAGACGACCTGCTGGAGAAAGCCGGAGCGCGGGCTCAGTCCGGGGGGCGGCGGCGCGGCTCCGGGGATGGCGGCGGCTCCGCTG
 CCTAGAACCTTGCTCTGCTGGACGACCTCTTCGGCCCTCGCGCCCCGAGTCAGCCCCCGCGCGCGCGCGCGAGGCCCTACCGCGCGCGAGGCGAC

AspLeuGlyThrArgArgProAlaGlyGluAlaGlySerAlaGlyLeuSerArgGlyAlaAlaAlaAlaAlaAlaProGlyMetAlaAlaAlaProLeu

B

[illegible]

LeuLeuLeuLeuValProValProLeuLeuProLeuLeuAlaGlnGlyProGlyGlyAlaLeuGlyAsnArgHisAlaValTyrTrpAsnSerSer
 A B B
 l s s
 w p p
 N M M
 1 1 1

A2R185.28

CCAACCAGCACCTCGCGCGAGAGGGCTACACCGTGCAGGTGAACGTGAACGACTATCTGTATATTACTGCCCGCACTACAACAGCTCGGGGGGTGGGCC
 GTGTGTCGTGGACCGCGCTCTCCGATGTGGCAGCTCCACTTGCCTGCTGATAGACCTATAAATGACGGCGTGATGTTGTGAGCCCCCACC0363

AsnGlnHisLeuArgArgGluGlyTyrThrValGlnValAsnValAsnAspTyrLeuAspIleTyrCysProHisTyrAsnSerSerGlyValGlyPro

A D AA
 v r pv
 a a aa
 1 2 11

A2R185.28

A	D	AA
v	r	pv
a	a	aa
1	2	11

CCAACCGACCTGCGGGCAGAGGGGCTACACCGTGCAGGTGAACGTGAACGACTATCTGTATATTTCCTGCCGCACTACACAGCTCGGGGGTGGGCCC
 201 GGTGTGTCGTGGACGCCCTCTCCCGATGTGGCAGCTCCACTTGCACCTGTCTGATAGACCTATAAATGACGGGGCGTGATGTTGTCTGAGCCCCACCCGGG 300
 AsnGlnHisLeuArgArgGluGlyTyrThrValGlnValAsnValAsnAspTyrLeuAspIleTyrCysProHisTyrAsnSerSerGlyValGlyPro -
 B B
 s s
 pB p
 B1sPSX B1sAB1P
 a2rsmm r rpa2s
 n8Faaa a Fan8s
 261111 2 11261
 ////
 CGGGGCGGGACCGGGCCCGAGGCGGGGCGAGCAGTACGTGCTGTACATGGTGAGCCGCAACGGCTACCGCAGCTGCAACGCCAGCCAGGGCTTCAAG
 101 GCCCCGCCCTGCCCCGGGCGCTCCGCCCCGCTCTCGTCATGCACGACATGTACCACTCGGCGTTGCGATGGCGTGGACGTTGCGGTCCGATCCGAAGTTC 400
 GlyAlaGlyProGlyProGlyGlyGlyAlaGluGlnTyrValLeuTyrMetValSerArgAsnGlyTyrArgThrCysAsnAlaSerGlnGlyPheLys -
 E
 C

E
 C
 o H
 4 a
 7 e
 3 2

E
 C
 o HS
 4 af
 7 ec
 3 21

H
 a
 e
 2

AZT7, 49

N
 g
 o
 M
 1

CCGTGGAGTGCAACCGCCCGCACGCGCGCACAGCCCCATCAAGTTCTCGGAGAAGTTCCAGCGCTACAGCGCCTTCTCTCTGGGCTACGAGTTCCAGG
 GCGAACCTCAAGTTGGCGGGCGTGCGCGCGGTGTCGGGGTAGTTCAAGAGGCTCTCAAGGTCGCGGATGTGCGCGGAGAGAGACCCCGATGCTCAAGGTGC

ArgTrpGluCysAsnArgProHisAlaProHisSerProIleLysPheSerGluLysPheGlnArgTyrSerAlaPheSerLeuGlyTyrGluPheHisAla

E
 S
 P

AZT7, 49

N
 9
 O
 M
 1

E S B
 a c s
 o a
 1 1
 CCGGCCACGAGTACTACTACATCTCCACGGGGG
 P X B
 M m s
 l n r
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0032

TITLE

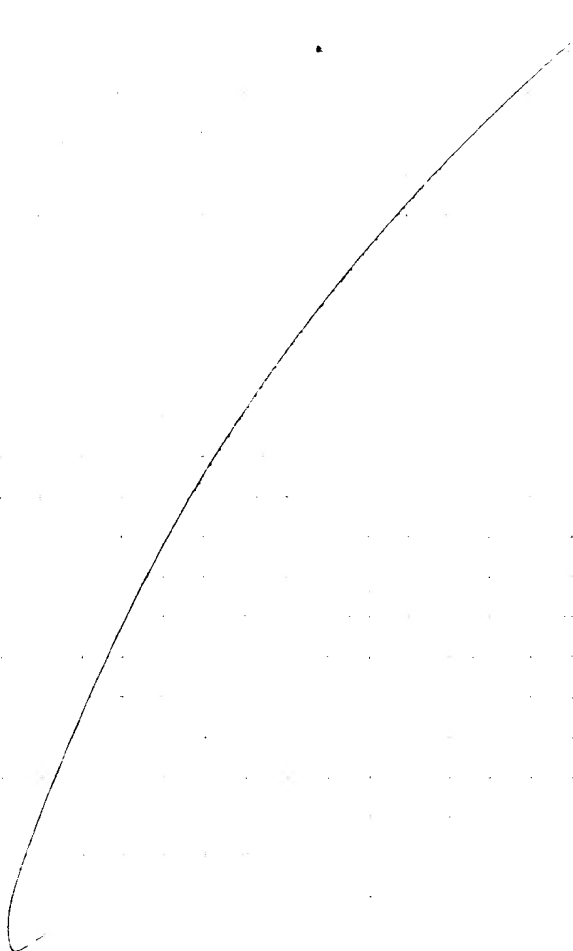
A2 S29.

Project No. _____

Book No. _____

62

From Page No. _____



To Page No. _____

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opc

Date

Invented by

Carl H. H. H.

Date

Recorded by

0033

(Line 7) (Six Base) MAP of: C6.Seq check: 9352 from: 1 to: 636
 H5AL 132-11, C6-no vector
 2491, T7, DPC3266, DPC3267, DPC3274, DPC3275
 SR1810 KOZLOSKY
 file: [BERTLESJ.HEKL]C6.SEQ

With 114 enzymes: *

12:12



100
 GCCAGACCAACCGGACCTCGGGGGGATGCGGCTGCTGCCCTCTGCGGACTGTCTCTGGGCGCGCTTCTCTGGGCTCCTCTCGCGGGGGCTCCA
 CGGTCTGGTCTGGGCTGGAGCCCCGCTACGCCGACGACGGGAGCAGCCTGACAGGAGACCCGCGCAAGGAGCGAGGGGAGACGCCCCCGAGGT
 AlaArgProAsnArgThrSerGlyAlaMetArgLeuLeuProLeuLeuArgThrValLeuTrpAlaAlaPheLeuGlySerProLeuArgGlySerSer

101
 GCCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

200
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

201
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

300
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

301
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

400
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

401
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

500
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

501
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

600
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

700
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

800
 CCGGCTCCGCCACGTAGTCTACTGGAACCTCCAGTAACCCAGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCTCAACGATTACCTAGACATTGTCTG
 CGGAGGCGGTGCATCAGATGACCTTGAGGTCTTGGGGTCCACGAAGCTCCTCTCGGCGACACCTCGACCCGAGGTGTCTAATGGATCTGTAACAGAC
 LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

TITLE

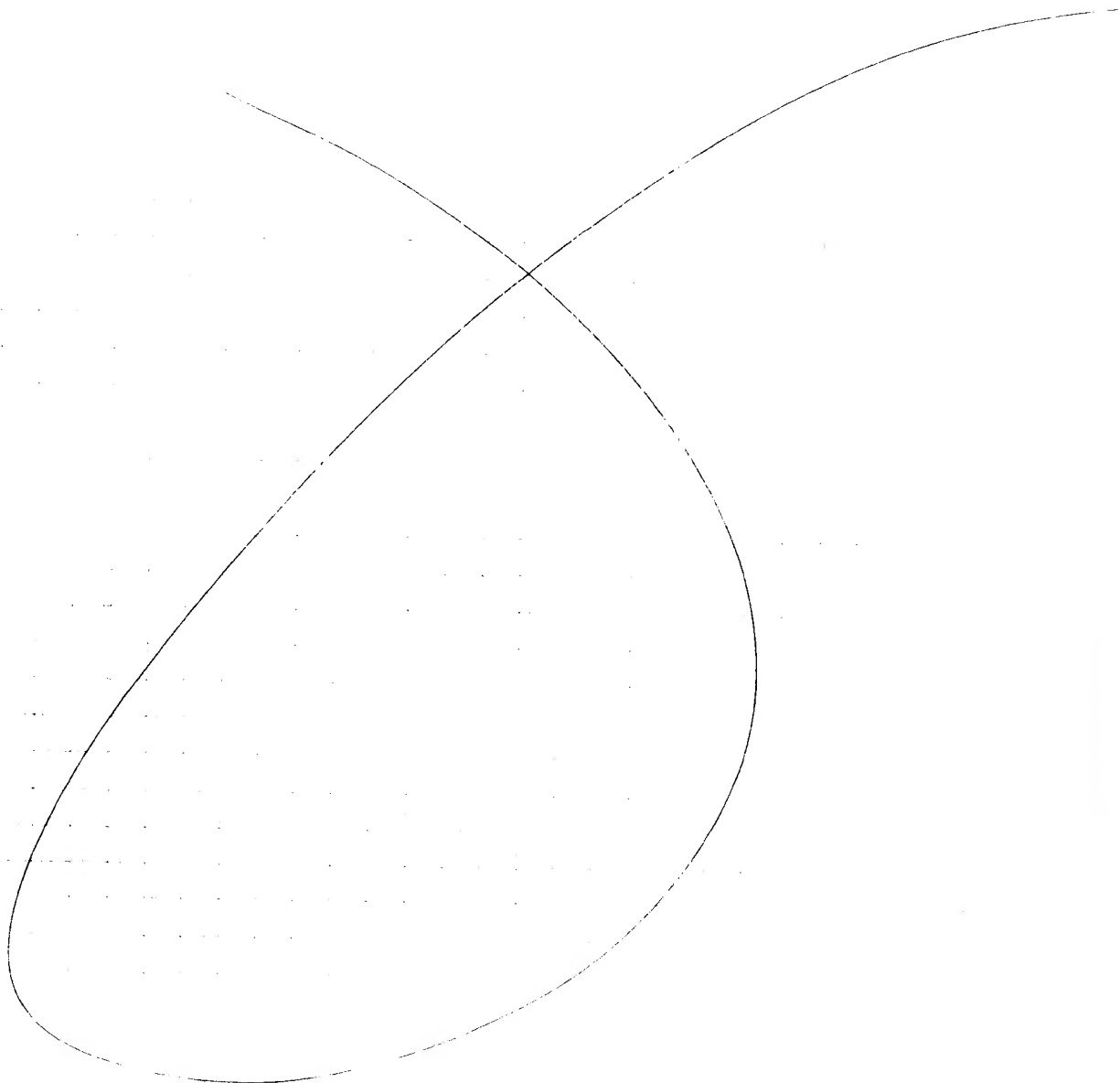
Cb Seq.

Project No. _____

Book No. _____

66

From Page No. ...



Witnessed & Understood by me,

opL

Date

Invented by

Recorded by

Carl Koster

Date

To Page No. _____

0035

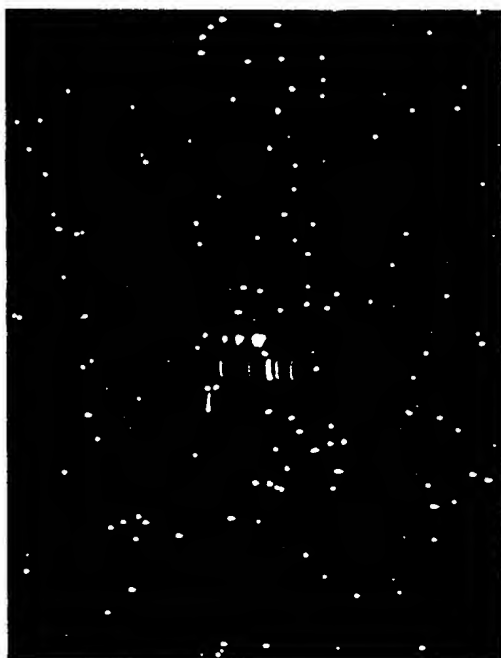
TITLE C6 T7 Ribo PCR

Project No. _____

Book No. _____

39

From Page No. _____



C6 Binding Region
T7 RNA Pol

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PPL

Date

Invented by

Recorded by

Carl Hopkins

Date

To Page No. _____

0036

TITLE Clb Nth

Project No. _____

Book No. _____

From Page No. _____

< 189 ~ 1.6Kb in HSB-2

cyk



new A2 T7
Riboprobe
Template

To Page No. _____

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[Signature]

Date

Invented by

Recorded by

Date

0037

Working File
Do Not Copy!

With 114 enzymes: *

13:38

E B
Ac s AAB1sSSX
po r vpa2rmrm
OR F aan8Fa1a
11 1 11261111
/ 1414
GAATTCGGGCCCCGGGCCAACGCTGACCGATACGCAGTCTACTGGAACCGTAGCAACCCAGGTTTCAGGTGAGCGCTGTGGGTGATGGCGGCGGCTATA
1 CTTAAGGCCCGGGCCCGGTGCGACTGGGTATGCGTCAGATGACCTTGGCATCGTTGGGGTCCAAAGTCCACTCGCGACACCCACTACCGCCGCCGATAT 100
a: GluPheArgAlaArgAlaAsnAlaAspArgTyrAlaValTyrTrpAsnArgSerAsnProArgPheGlnValSerAlaValGlyAspGlyGlyGlyTyrThr -

B
P
u
1
1
0
2
1
D s a l
E c o R S
B N
BsKNH s
aaaaa p
nHsre B
11112 2
//
101 CCGTGGAGGTGAGCATCAACGACTACCTGGATATCTACTGCCACACTACGGGGCGCCGCTGCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTACAT 200
GGCACCCTCCACTCGTAGTTGCTGATGGACCTATAGATGACGGGTGTGATGCCCGCGCGGACGGGGCGGACTCGCGTACCTCGCCATGTAGGACATGTA
a: ValGluValSerIleAsnAspTyrLeuAspIleTyrCysProHisTyrGlyAlaProLeuProProAlaGluArgMetGluArgTyrIleLeuTyrMet -

E
c
o H
4 a
7 e
3 2
B
s
s
m
1
B P
AsHSX Dp P
vramm ru s
aFeaa aM s
11211 21 1
////
201 GGTGAATGGTGAAGGCCACGGCTCTGTGATGACGGGACGGAGGCTTCAAGGCTGGGAATGCAACCGGCCCGAGCGCCCGGGGACCCCTCAAGTTC 300
CCACTTACCACCTCCCGGTGCGGAGGACACTGGTGGCCGTCGCTCCGAAGTTCGGGACCCCTACGTTGGCCGGGCGTCGCGGGCCCCCTGGGGAGTTCAAG
a: ValAsnGlyGluGlyHisAlaSerCysAspHisArgGlnArgGlyPheLysArgTrpGluCysAsnArgProAlaAlaProGlyGlyProLeuLysPhe -

E
a
r
l
E B
a a
e l
1 1
B
s
t
X
1
301 TCAGAGAAGTTCCAACTCTTACCCCCCTTTCCCTGGGCTTTGAGTTCGGCCCTGGGCCACGAATACTACTACATCTCTGCCACACCTCCCAACCTCGTGG 400
AGTCTCTTCAAGGTTGAGAAGTGGGGAAAGGGACCCGAAACTCAAGGCCGACCGGTGCTTATGATGATGTAGAGACGCTGTGGAGGTTGGAGCACC
a: SerGluLysPheGlnLeuPheThrProPheSerLeuGlyPheGluPheArgProGlyHisGluTyrTyrTyrIleSerAlaThrProProAsnLeuValAsp -

B
s
p
B1
a2
n8
26
/

B B E
s p c
s p o
1 1 i

A N
1S Ps
wf sp
Nc tB
11 12

401 ACCGACCCTGCTGCGACTCAAGGTTTATGTGCGTCCAACCAATGAGACCTGTATGAGGCTCCAGAGCCCATCTTCACCAGTAACAGCTCCTGCAGCGG 500
TGGCTGGGACGGAGCTGAGTTCCAAATACACGAGGTTGGTTACTCTGGGACATATCCGAGGTCTCGGGTAGAAGTGGTCATTGTGAGGACGTCGCC
a: ArgProCysLeuArgLeuLysValTyrValArgProThrAsnGluThrLeuTyrGluAlaProGluProIlePheThrSerAsnSerSerCysSerGly -

B
a
l
1
E
a
r
l
B
s
p
B1
a2
n8
26
/
501 CCTGGGTGGCTGCCACCTCTTCTCACCACCGTCCCTGTGCTGTGGTCCCTTCTGGGCTCCTAGTGTGAGCCGGAGAACACCAGCCCCACCTGGACCCC 600
GGACCCACCGAGCTGGAGAAGGAGTGGTGGCAGGGACACGACACCGAGGAAGACCGAGGATCACAGTCCGGCCTCTTGTGGTGGGGTGGACCTGGGG
a: LeuGlyGlyCysHisLeuPheLeuThrThrValProValLeuTrpSerLeuLeuGlySerEndCysGlnAlaGlyGluHisGlnProHisLeuAspPro -

0038

B
D Es
s ap
a eM
l ll

601 GTGACCTTTGCCCTCTGACCTGCCACGGCCACCTCCGAGACAAAATCCTTGCTGCTTCTTTTCATGGTGTGTCCCGCCGGAGGAGGCCATCCATCCGT
CACTGGAAACGGGAGACTGGACGGTGGCGGTGGAGGCTCTGTTTTAGGAACGACGAAGAGAAAGTACCACGACAGGGCGGCCTCTCCGGTAGGTAGGCA 700

a: ValThrPheAlaLeuEndProAlaThrAlaThrSerGluThrLysSerLeuLeuLeuLeuPheHisGlyAlaValProProGluGluAlaIleHisProSer -

P B
Dp P s
u s 3 B S
aM s 6 b t BS
21 l 1 s y gf
/ li
/ ll

701 CCCTGGGATGCAACATGGGGTCCCAATGCCTGAGGAGAAGACCCCCCCCCAAGGCTGACTCGCTTTCACCAGGGCCACCAGGGCCATCCAGTGTGYATA
GGGACCCTACGTTGTACCCAGGGTTACGGACTCCTCTTCTGGGGGGGGTTCGACTGAGCGAAAGTGGTCCCGGTGGTCCCGGTAGGTACACACRTAT 800

a: LeuGlyCysAsnMetGlySerGlnCysLeuArgArgArgProProProLysAlaAspSerLeuSerProGlyProProGlyProSerSerVal???End -

ATTCTTT
801 ----- 807
TAAGAAA

a: PhePhe -

Enzymes that do cut:

AccI	AlwNI	ApoI	ApaI	AvaI	BalI	BanI	Ban2	BbsI	BglI	Bpu1102I	BpmI	BsaI
BsaH1	BsmI	Bsp1286	BspMI	BsrFI	BstXI	Bsu36I	Dra2	DsaI	EaeI	EarI	Eco473	EcoNI
EcoRI	EcoR5	Hae2	KasI	NarI	NspB2	PpuMI	PssI	PstI	SfcI	SfiI	SmaI	SrfI
StyI	XmaI											

Enzymes that do not cut:

Aat2	Afl2	Afl3	AgeI	ApalI	AscI	AseI	Asp718	Asu2	Avr2	BamH1	BcgI	BclI
Bgl2	BsaA1	BsaB1	BsiE1	BsiW1	BspE1	BspH1	BssH2	Bst1107	BstE2	Clal	DraI	Dra3
Drd1	Eam1105	Eco571	Esp31	FspI	HgiA1	Hinc2	Hind3	HpaI	KpnI	MluI	MunI	NcoI
NdeI	NgoM1	NheI	NotI	NruI	NsiI	NspH1	PacI	PflMI	PmeI	PmlI	PvuI	Pvu2
Rsr2	SaiI	Scal	SgrA1	SnaB1	SpeI	SphI	Sse8387	SspI	SstI	Sst2	StuI	SwaI
Tth31	Tth32	XbaI	XcmI	XhoI	Xho2	Xma3	XmnI					

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

TRANSLATE of: a2.seq check: 6473 from: 83 to: 796
generated symbols 1 to: 238.

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Quality: 137.9 Length: 246
Ratio: 0.741 Gaps: 6
Percent Similarity: 67.416 Percent Identity: 48.876

```

1 .....RARANADRYAVYWNRSNPRFQVSAVG 26
      :|::|.|||||.||:::
1  MAAAPLLLLLLLVPVPLLPLLAQGPGGALGNRHAVYWNSSNQHLRR... 46
27 DGGGYTVEVSINDYLDIYCPHY.....GAPLPPAERMERYILYMVNGE 69
      :||||:|.:||||| |||. |:| |. |||. :
47 ..EGYTQVQNVNDYLDIYCPHYNSSGVGPGAGPGPGGGAEQYVLYMVS RN 94
70 GHASCDHRQRGFKRWE CNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHE 119
      |. |:| | ||||| || |:| ||||| ::|||:| |:| |
95 GYRTCNASQ.GFKRWE CNRPHAPHSP IKFSEKFQRYSAFSLGYEFHAGHE 143
120 YYYISATPPNLVDRPCLRLKVYV.....RPTNETLYEAP EPIFTSNSSC 163
      |||| | |:| : :|||:| |:| : : : :| :| :| :|
144 YYYIS.TPTHNLHWKCLRMKV FVCCASTSHSGEKPVP TLPQFTMGPNVKI 192
164 SGLGGCHLFLTTPVVL.WSLLGS*..... 186
      ..|::| . ||| |:|.
193 NVLEDFEGENPQVPKLEKSISGTSPKREHLPLAVGIAFFLMTFLAS 238

```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.

WORKING FILE
DO NOT COPY!

to: C6.Pep check: 8194 from: 1 to: 201

TRANSLATE of: c6.seq check: 6086 from: 53 to: 655
generated symbols 1 to: 201.

HEKL 132-11, C6-no vector
2491,T7,DPC3266,DPC3267.DPC3274,DPC3275
SR1810 KOZLOSKY
file: [BERTLESJ.HEKL]C6.SEQ . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396

Quality:	118.5	Length:	216
Ratio:	0.637	Gaps:	7
Percent Similarity:	61.988	Percent Identity:	46.199

Mlerk6.Pep x C6.Pep 16:31 ..

```
1 .....RARANAD.RYAVYWNRSNPRFQVSAVGDGGGY 31
      . |:. . . |. . . . . |. . . . . |:
1 MRLPLLRRTVLWAAFLGSPLRGGSSLRHVYWNSSNPRL.....RGDA 44
      .
32 TVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCD.HRQRG 80
      .||: . . . . . |||: . . . . . | : |||: . |. . . . . |:
45 VVELGLNDYLDIVCPHYEGPGPPEGP.ETFALYMVDWPGYESCQAEGPRA 93
      .
81 FKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNL 130
      :||| | . | |. . . |||: | ||| . . . . . ||. . . . . |:
94 YKRWVC...SLPFGHVQFSEKIQRFTPFSLGFEFLPGETYYYISVPTPES 140
      .
131 VDRPCLRLKVYVRPTNETLYEAPEPIFTSNSSCSGLGGCH..... 170
      :. ||| . | | . . . . |. . . . . :||: . |. .
141 SGQ.CLRLQVSVCCKERKSESAHPVGSPGESGTSGWRGGDTPSPLCLLLL 189
      .
171 LFLTTPVPLWSSLGS* 186
      |:| . . . . . |: |
190 LLLLIILRLRIL.... 201
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: Elkl.Pep check: 1665 from: 1 to: 240

TRANSLATE of: tele7.seq check: 2210 from: 308 to: 1345
generated symbols 1 to: 346.
[hollingsworth.tele7] ELKL-E7.SEQ + ELKL-E7-3PRIME.SEQ;
req#1262
mGel 97 #2491+ #2492-/ mGel101 DPC2236+ DPC2239+/ mGel104 DPC2258+
DPC2257-/mGel105 DPC2261- /mGel107 DPC2271+ 2272- 2273- 2274+ . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396

Quality:	82.7	Length:	248
Ratio:	0.445	Gaps:	6
Percent Similarity:	46.067	Percent Identity:	28.652

Mlerk6.Pep x Elkl.Pep 16:46 ..

```
1 RARANADR.....YÄVYWNRSNPRFQVSAVG.....DGGGY 31
. || : . : : : : : : : : : : : : : : : : . | | .
1 MARPGQRWL GKWL VAMVWALCRLATPLAKNLEPVSWSSLNPKFLSGKGL 50
32 TVEVSINDYLDIYCPHYGAPLPPAERMERYILYMNVEGHASCDHRQRF 81
.: .|. | | | | | : : | . | | | | : | . | . | .
51 VIYPKIGDKLDIICPRAEAGRP....YEYYKLYLVRPEQAAACSTVLDPN 96
82 KRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNLV 131
. | | | | : : : : | . | | | | : : | | : : | : | . | . . |
97 VLVTCNR...PEQEIRFTIKFQEFSPNYMGLEFKKHHDYYITSTSNGSLE 143
132 D.....RPCLRLKVYVRPTNETLYEAPEPIFTSNSSCSGLGGCHLFL 173
: . : : : : : : : : : : : : : : : : : : : : : : : :
144 GLENREGGVCRTMTMKIIMKVGQDPNAVTPQLTTSRPSKEADNTVKM.A 192
174 TTVPVLWSLLGS*..... 186
| . | . : : | | .
193 TQAPGSRGSLGSDGKHETVNQEEKSGPGASGGSSGDPDGGFFNSKVAL 240
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.

WORKING FILE
DO NOT COPY!

to: Lerk5.Pep check: 8553 from: 1 to: 240

TRANSLATE of: lerk5.leg check: 889 from: 1 to: 1002
generated symbols 1 to: 334.
Coding region of human LERK-5.

Symbol comparison table: Gcgc core disk:[Gcgc core.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396

Quality:	83.2	Length:	250
Ratio:	0.447	Gaps:	5
Percent Similarity:	47.727	Percent Identity:	27.841

Mlerk6.Pep x Lerk5.Pep

16:59 ..

```
1 .....RARANADRY....AVYWNRSNPRFQVSAVGDG 28
      .|.. ..: :|||.||.:| .|
1 MAVRRDSVWKYCWGVLMLVLCRTAISKSIIVLEPIYWNSSNSKFL.....PG 45
29 GGYTVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQ 78
      .|...: .|.| |||.||.:. . . . .| | :|||: :. .|.:.:
46 QGLVLYPQIGDKLDIICPKVDS..KTVGQYEYYKVYMVDKDQADRCTIKK 93
79 RGFKRWE CNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYYISATPP 128
      . . :| | | : . :||. ||| |. | :|:| | . . :| | | . .
94 ENTPLLNC...AKPDQDIKFTIKFQEFSPNLWGLEFQKNKDYYIISTSN 140
129 NLVD.....RPCLRLKVY 141
      .| : || |
141 SLEGLDNQEGGVCQTRAMKILMKVGQDASSAGSTRNKDPTRRPELEAGTN 190
142 VRPTNETLYEAP EPIFTSNSSCSGLGGCHLFLTTVPVLWSSLGS*..... 186
      .|.....: | :| . . . . .| :| : : .| : : : : :
191 GRSSTTSPFVKPNPGSSTDGNSAGHSGNNILGSEVALFAGIASGCIIFIV 240
```

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

TRANSLATE of: b61.seq check: 6304 from: 74 to: 688
generated symbols 1 to: 205.

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

```

      Quality: 128.5      Length: 212
      Ratio: 0.691      Gaps: 4
Percent Similarity: 59.218  Percent Identity: 45.251

```

```

1 .....RARANADRYAVYWNRSNPRFQVSAVGDDGGGYTVEVSIN 38
      . |. |||. |: |||. |||:|. .::||:|. | :|
1 MEFLWAPLLGLCCSLAAADRHTVFWNSSNPKFR.....NEDYTIHVQLN 44
39 DYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQRGFKRWECNR 88
      ||: |||. ||||:.. .:. .|||. ||||: |: |:.. |:.. :: ||: |||
45 DYVDIICPHYEDHSVADAAMEQYILYLVHEHEEYQLCQPQSKDQVRWQCNR 94
89 PAAPGGPLKFSEKFQLETPFSLGFEEFRPGHEYYYISATPPNLVDRPCLRL 138
      |.|. || |: |||| | |||. || ||:|. ||. |||||. . . || ||||
95 PSAKHGPEKLSEKFQRFPTFTLGKEFKEGHSYYYISKPIHQHEDR.CLRL 143
139 KVVYVRP.....TNETLYEAPEPIFTSNSSCSGLGGCHLF.LTTV 176
      || |.. .|. .|. |:| . |.: ::::|| |. .
144 KVTVSGKITHSPQAHVNPQEKRLAADDPEVRVLHSIGHSAAPRLFPLAWT 193
177 PVLWSLLGS*... 186
      .: |:|. ||
194 VLLLPLLLLQTP 205

```


GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558

generated symbols 1 to: 186.

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to: Mc6.Pep check: 7024 from: 1 to: 168

TRANSLATE of: mc6.seq check: 5844 from: 2 to: 505

generated symbols 1 to: 168.

Sequence of murine C6 (LERK-4) as derived from the genomic clone (3.5 kbp Sst1 fragment).

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396

Quality:	111.3	Length:	196
Ratio:	0.663	Gaps:	7
Percent Similarity:	65.190	Percent Identity:	45.570

Mlerk6.Pep x Mc6.Pep

16:31 ..

```
1 RARANADRYAVYWNRSNPRFQVSAVGDGGGYTVEVSINDYLDIYCPHYGA 50
      :...|| ||::|||||:||||:
1 .....LLRGDAV.....VELGFNDYLDIFCPHYES 25
51 PLPPAERMERYILYMVNGEG.HASCDHRQRGFKRWECNRPAAPGGPLKFS 99
  | ||:. | : |||: .| .|:... .:|.||:|..| || :|:|
26 PGPPEGP.ETFALYMVDWSGYEACTAEGANAFQRWNCMPFAPFSPVRES 74
100 EKFOLETPFSLGFEFRPGHEYYYISATPPNLVDRPCLRLKVYVRPTN.ET 148
  ||:| :|||.|||| | |..|||||...|: .:| ||||. | | ..: .
75 EKIQRYPFPLGFEFLPGETYYYYISVPTPESPGR.CLRLQVSVCKESGS 123
149 LYEAEPEI.FTSNSSCSGLGGCH.....LFLTTPVVLWSSLGS* 186
  .|:.|: .:|.||: |. | :|:|: | .
124 SHESAHPVGSPGESGTSGWRGGHAPSPLCLLLLLLLLPILRLRLVL. 168
```

GAP of: Mlerk6.Seq check: 8999 from: 1 to: 797

WORKING FILE
DO NOT COPY!

to: A2.Seq check: 9214 from: 1 to: 987

HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL]

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgapdna.Cmp
CompCheck: 6876

Gap Weight: 5.000 Average Match: 1.000
Length Weight: 0.300 Average Mismatch: 0.000

Quality: 362.8 Length: 1011
Ratio: 0.455 Gaps: 9
Percent Similarity: 56.016 Percent Identity: 56.016

Mlerk6.Seq x A2.Seq 16:33 ..

```

      .
      .
      .
1 .....CGGGCCCGGGCCAACGCTGAC 21
      | | | | |
101 TGCCGCTGCTGCCGCTGCTGGCCCAAGGGCCCGGAGGGGCGCTGGGAAAC 150
      .
22 CGATACGCAGTCTACTGGAACCGTAGCAACCCCAGGTTTCAGGTGAGCGC 71
   || | || | | | | | | | | | | | | | | | | | | |
151 CGGCATGCGGTGTACTGGAACAGCTCCAACCAGCACCTGCGG..... 192
      .
72 TGTGGGTGATGGCGGCGGCTATACCGTGGAGGTGAGCATCAACGACTACC 121
   | | | | | | | | | | | | | | | | | | | | |
193 .....CGAGAGGGCTACACCGTGCAGGTGAACGTGAACGACTATC 232
      .
122 TGGATATCTACTGCCACACTA.....CGGGGCG 150
   | | | | | | | | | | | | | | | | | | | | |
233 TGGATATTTACTGCCCGCACTACAACAGCTCGGGGGTGGGCCCCGGGGCG 282
      .
151 CCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTACATGGTGAA 200
   | | | | | | | | | | | | | | | | | | | | |
283 GGACCGGGGCGCGGAGGCGGGGCAGAGCAGTACGTGCTGTACATGGTGAG 332
      .
201 TGGTGAGGGCCACGCCTCCTGTGACCACCGGCAGCGAGGCTTCAAGCGCT 250
   | | | | | | | | | | | | | | | | | | | | |
333 CCGCAACGGCTACCGCACCTGCAACGCCAGCCAG...GGCTTCAAGCGCT 379
      .
251 GGAATGCAACCGGCCCGCAGCGCCCGGGGACCCCTCAAGTTCTCAGAG 300
   | | | | | | | | | | | | | | | | | | | | |
380 GGGAGTGCAACCGGCCGCACGCCCCGCACAGCCCCATCAAGTTCTCGGAG 429
      .
301 AAGTTCCAACCTCTTACCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGG 350
   | | | | | | | | | | | | | | | | | | | | |
430 AAGTTCCAGCGCTACAGCGCCTTCTCTCTGGGCTACGAGTTCCACGCCGG 479
      .
351 CCACGAATACTACTACATCTCTGCCACACCTCCCAACCTCGTGGACCGAC 400
   | | | | | | | | | | | | | | | | | | | | |
480 CCACGAGTACTACTACATCTCCACGCCCACTACAACC...TGCACTGGA 526

```

0046

Quality: 183.3 Length: 338
 Ratio: 0.554 Gaps: 3
 Percent Similarity: 61.846 Percent Identity: 61.846

Mlerk6.Seq x Mc6.Seq

14:13 ..

```

99 TACCGTGGAGGTGAGCATCAACGACTACCTGGATATCTACTGCCCACACT 148
   ||||| || || ||||| ||||| ||||| ||||| |||||
20 .GTGGTGGAGCTGGGCTTCAACGATTACCTAGACATCTTCTGCCCACATT 68
   || || || ||||| || || || || || || || || ||
149 ACGGGGCGCCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTAC 198
   || || || ||||| || || || || || || || || ||
69 ATGAAAGCCCAGGGCCCC...CAGAAGGCCCGGAAACCTTTGCATTATAC 115
   ||||| || || ||||| ||||| ||||| ||||| |||||
199 ATGGTGAATGGTGAGGGCCAC...GCCTCCTGTGACCACCGGCAGCGAGG 245
   ||||| || || ||||| ||||| ||||| ||||| |||||
116 ATGGTGGACTGGTCAGGCTACGAGGCCCTGCACGGCAGAGGGGGCAAATGC 165
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
246 CTTCAAGCGCTGGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCA 295
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
166 CTTCCAGCGCTGGAATTGCTCGATGCCTTTTGGCCCTTTTCAGCCCTGTTC 215
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
296 AGTTCTCAGAGAAGTTCCAACCTCTTCACCCCTTTTCCCTGGGCTTTGAG 345
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
216 GATTCTCAGAAAAGATTCAGCGCTACACACCCTTCCCGCTGGGCTTTGAG 265
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
346 TTCCGGCCTGGCCACGAATACTACTACATCTCTGCCACACCTCCCAACCT 395
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
266 TTCTTGCTGGAGAGACTTACTACTACATCTCGGTGCCGACTCCGGAGAG 315
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
396 CGTGGACCGACCCTGCCTGCGACTCAAGGTTTATG... 430
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
316 TCCTGgCCG...GTGCCTGAGACTCCAGGTGTCTGTCT 350

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\$

Quality: 104.9 Length: 411
Ratio: 0.373 Gaps: 1
Percent Similarity: 39.858 Percent Identity: 39.858

Mlerk6.Seq x Lerk5.Seq

14:01 ..

```

      .
      .
      .
140 .....GCCCCACTACGGGGCGCCGCTGCCCCCGGCTGAGCG 176
      |   |||   ||   |||||   || |
1890 ATACCCACAGATAGGAGACAAATTGGATATTATTTGCCCCAAAGTGGACT 1939
      .
177 CATGGAGCGGTACATCCTGTACATGGTGAATGGTGAGGGCCACGCTCCT 226
      | | | | | | | | | | | | |
1940 CTAAAACTGTTGGCCAGTATGAATATTATAAAGTTTATATGGTTGATAAA 1989
      .
227 GTGACCACCGGCAGCGAGGCTTCAAGCGCTGGGAATGCAACCGGCC.... 272
      | | | |||   || | | | | | | | |
1990 GACCAAGCAGACAGATGCACTATTAAGAAGGAAAATACCCCTCTCCTCAA 2039
      .
273 ...CGCAGCGCCCCGGGGGACCCCTCAAGTTCTCAGAGAAGTTCCAACCTCT 319
      ||   || | | | | | | | | | | |
2040 CTGTGCCAAACCAGACCAAGATATCAAATTCACCATCAAGTTTCAAGAAT 2089
      .
320 TCACCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGGCCACGAATACTAC 369
      ||| |||   | ||| | || | | | | | | |
2090 TCAGCCCTAACCTCTGGGGTCTAGAATTTCAGAAGAACAAGATTATTAC 2139
      .
370 TACATCTCTGCCACACCTCCCAACCTCGTGGACCGACCTGCCTGCGACT 419
      || ||| | || | | | | | | | |
2140 ATTATATCTACATCAAATGGGTCTTTGGAGGGCCTGGATAACCAGGAGGG 2189
      .
420 C..... 420
2190 AGGGGTGTGCCAGACAAGAGCCATGAAGATCCTCATGAAAGTTGGACAAG 2239
      .

```

HEK/ELK Meeting Minutes

B. Davison in the absence of Doug Cerretti.

Nicole Nelson summarized the recent screening of a murine embryonic cDNA library with a combination of LERKs(A2,C6) plus GSK beta kinase(Fred Fletcher's probe): Hybridization conditions were 42 C in 50% Stark's followed by washes at 63 C, 0.1X SSC.

13 initial positives were obtained of which 4 did not repeat; Nicole will rescreen these using a less stringent wash protocol. Currently, the sequence analysis indicates that the collection contains at least 1 gsk Beta clone, 1 gsk Beta-like clone and interestingly, a new LERK, LERK-6.

All four cysteine residues are conserved while inspection of the carboxy terminal portion of the sequence indicates that LERK-6 fits into the GPI-linked class(similar to LERKS 1,3 and 4). The amino terminus of the protein is apparently lacking about 20 or 25 amino acid residues in the current clone. In the binding region, the LERK-6 DNA sequence displays about 70% identity with A2(LERK 3) corresponding to 288 bp of overlap.

Efforts are now being directed at identification of a source to obtain the human homologue.

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Immunex Corporation
Attention: Stephen L. Malaska
Legal Affairs Department
51 University Street
Seattle, WA 98101

Deposited on Behalf of: Immunex Corporation (Docket No. 2826)

Identification Reference by Depositor:

ATCC Designation

Recombinant phage lambda gt10 vector,
clone lambda 13M LERK-6 (murine)

75829

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description
indicated above.

The deposit was received
accepted.

by this International Depository Authority and has been

AT YOUR REQUEST:

☒ We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right
to receive, or if a U.S. Patent is issued citing the strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your
responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period
of at least five years after the most recent request for a sample. The United States and many other
countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested
viable.

On that date, the culture was

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Bobbie A. Brandon Date:
Bobbie A. Brandon, Head, ATCC Patent Depository

Form BP4/9

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